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Full Name of Author — Nom complet de l'auteur

JAMES, David B.

Date of Birth — Date de naissance
9TH MAY 1945.

Country of Birth — Lieu de naissance
AUSTRALIA.

Permanent Address — Résidence fixe

29 Lane Street
Perth 6000
West Australia

Title of Thesis — Titre de la thèse

Manual Soft Tissue Manipulation of Traumatized Skeletal Rabbit Muscle

University — Université

University of Alberta

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée

Master of Science

Year this degree conferred — Année d'obtention de ce grade
Spring, 1981

Name of Supervisor — Nom du directeur de thèse
Dr. S. Kumar

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MANUAL SOFT TISSUE MANIPULATION OF TRAUMATIZED SKELETAL RABBIT MUSCLE

by

© DAVID B. JAMES

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PHYSICAL THERAPY

EDMONTON, ALBERTA

SPRING, 1981

THE UNIVERSITY OF ALBERTA

RELEASE FORM

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YEAR THIS DEGREE GRANTED 1981

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(Signed) David James

PERMANENT ADDRESS:

29 Lane Street

Perth 6000

West Australia

DATED December 22, 1980

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled MANUAL SOFT TISSUE MANIPULATION OF TRAUMATIZED SKELETAL RABBIT MUSCLE submitted by DAVID B. JAMES in partial fulfillment of the requirements for the degree of Master of Science.

A Kumar
Supervisor

R B Stein

David C. Simpson

John F. Kramer

Date DECEMBER 22, 1980

DEDICATION

To my wife Erin

ABSTRACT

Seven large white male New Zealand rabbits were selected for investigation.

The study examined the effects of soft tissue manual therapy on injured gastrocnemius of rabbits over a period of twenty-one days after injury. The problem was to demonstrate any healing effect of daily treated muscle as compared to untreated muscle. The site of injury was limited to the medial head of gastrocnemius. Each rabbit was injured with a direct force under anaesthesia; both hind legs were injured, one randomly chosen leg was treated, the other acted as its control for each rabbit.

It was necessary to design an instrument to sufficiently traumatize the site only, and cause closed trauma. Further, a method of objectively recording the "dosages" of treatment with regard to the technique was devised so that treatments were reproducable.

When taking the tissue for histology on the appropriate designated days, it was possible through initial measurements taken prior to injury, to localize the epicentre of what was the trauma site, and to take the tissue from this impact area for subsequent histological investigation. Longitudinal sections of all of the tissues were prepared and stained with haematoxylin and eosin, to examine regeneration of new muscle, and with trichrome to evidence fibroblastic activity and

collagen connective tissue proliferation consistent with scar formation.

It was concluded that repair of skeletal muscle in experimental animals followed a pattern and progression similar to human skeletal muscle. Further, the treatment resulted in a more complete repair.

ACKNOWLEDGEMENTS

I returned and saw under the sun, that the race is not to the swift, or the battle to the strong, neither yet bread to the wise, not yet riches to men of understanding, not yet favor to men of skill; but time and chance happeneth to them all (Ecclesiastes II, 9:11).

There are numerous people who helped this undertaking come to fruition. I would like to thank my supervisor, Dr. Schrawan Kumar, and my examining committee: Dr. David Secord; Dr. Richard Stein; and Dr. John Kramer. An extra note of appreciation to Dr. Secord for his supportive, mature and warm assistance.

I also wish to acknowledge the following for their assistance throughout this endeavour:

- Arthur Stansfield, Histology Department, University of Alberta Hospital, for the preparation of histological slides; and Dr. Chansuria, Histopathology at S.M.R.I. for his help with the photography;
- Mr. Ted Germaine and his technical staff at S.M.R.I. for their valued assistance;
- Mr. Morris Trakk, who sacrificed the animals after completion of this study;
- "Edith" for her preparation of electron miscroscope grids for further study;
- Mr. Bob Konzik in Mineral and Mining Engineering, who constructed the traumatizing equipment;
- Clara Gallagher, who not only typed the manuscript but gave professional advice on many of its aspects, and was most supportive.

My thanks to the W.A.I.T. study leave committee in Australia for the leave of absence, and Dr. Lance Twomey, head of the Department of Physiotherapy, for his support.

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Chapter 1

THE PROBLEM

Introduction

The technique of soft tissue manipulation is frequently used to aid healing of traumatized skeletal muscle (Cyriax, 1969). Although a clinically effective technique, the skeletal muscle tissue response to such manipulation has not been examined in a controlled manner and documented. The process of skeletal muscle degeneration and subsequent regeneration has been documented over the past one hundred years (Allbrook and Aitken, 1951; Blontoff and Walker, 1960; Denny-Brown, 1951; Godman, 1957; LeGros, 1946); however, this process has only been histologically examined within the last thirty years (Bischoff, 1972, 1975; Church, 1970; Church, Noronha, and Allbrook, 1966; Järvinen, 1975; Mauro, 1961; Muir, Kanji, and Allbrook, 1965; Williams and Goldspink, 1971). The effects of the therapeutic intervention on the process, in particular soft tissue manual manipulative therapy, have not been investigated. In view of the frequency with which soft tissue manipulation is used by clinicians in the belief that muscle tissue regeneration is being influenced and the lack of scientific attention to this technique, it is imperative that research be undertaken. Such research has practical clinical implications with regard to effectiveness of treatment and

validation of treatment methodologies.

Objective of the Study

The objective was to determine if a reliably administered measured "dosage" of soft tissue manipulation applied to traumatized skeletal muscle enhances healing rate, as compared to no treatment.

Significance of the Study

Manual soft tissue manipulation is frequently used in the treatment of skeletal muscle trauma in conjunction with electrotherapeutic and other therapeutic modalities. With regard to closed skeletal muscle trauma, clinicians require an accurate knowledge of the normal process and rate of regeneration. Further to this, the effect of a given "dosage" of localized manual treatment on the process and quality of repair needs to be known.

Judicious and progressive treatments require objective measurement of the pressure applied to the healing tissues by the use of the hands, and the frequency, duration and progression of such treatments. Current methodologies lack objectivity and are thus difficult to reproduce and standardize.

The contribution of a study of the effect of manual manipulative therapy on rabbit skeletal muscle is that such investigation allows for a comparison of treated versus untreated muscle healing following measured, standardized, closed trauma. The administration of a set "dosage" to one

of the rabbit limbs while the other equally injured limb is untreated allows for comparisons between normal healing and the intervention into the normal process.

Ethical considerations prevent the infliction of trauma to human living skeletal muscle and its subsequent removal for histological investigation following trauma. Implications for the manual treatment of human skeletal muscle trauma may not be made from rabbit skeletal muscle investigation to human gastrocnemius tissue. However, without such descriptive information, the clinician will continue to act on the basis of belief, rather than fact.

Delimitations

1. The investigation was restricted to seven male rabbits of similar age and weight treated for periods stated following trauma.

2. Investigation was restricted to the region of the mid-belly of the medial gastrocnemius muscle.

3. Soft tissue trauma was restricted to a single technique of production.

4. Treatment was restricted to the application of measurable "dosages" of soft tissue manipulation as the only treatment administered.

5. Analyses of outcomes were restricted to examination of suitably stained histological preparations of the tissues.

Limitations

1. The degree of physical activity of the rabbits was individual and could only be partially controlled by cage confinement.

2. The rabbits' individual conscious response to, and tolerance of, the manual therapy to the injured muscle could not be controlled, but could be allowed for by ceasing treatment on limb withdrawal and recommencing after the limb was relaxed.

Operational Definitions

Closed Trauma - A physical injury brought about by the application of force to the tissues in such a way that the integrity of the skin remains intact.

Dosage - The measured pressure of application of the manual therapy, frequency of manipulating oscillating pressures, and duration of such application for each treatment.

Histological Investigations - The preparation of parafin wax embedded six μ thick longitudinal sections of the traumatized muscle. Such sections were stained with haematoxylin, eosin and trichrome.

Injured Control - The untreated leg which received an equal magnitude of injury as did the injured leg in the same rabbit.

Injured Treated Limb - That gastrocnemius medial head which received daily treatment from forty-eight hours post injury.

Normal Control - The tissue sample taken from a rabbit whose tissue had not been injured nor interfered with in any way (rabbit #7).

Site of Trauma - Includes skin, subcutaneous tissue, medial head of gastrocnemius, and other posterior compartment muscles in the calf complex. Included in the site of trauma are nerves and vessels in the immediate area.

Soft Tissue Manipulation - The use of fingers and thumbs to apply standardized rhythmical pressure to relaxed skeletal muscle.

Chapter 2

LITERATURE REVIEW

The histological study of skeletal muscle probably began with the work of Leeuwenhoek (cited in Mauro, 1979), who described the gross features of mouse skeletal muscle, under the microscope. The first detailed description of the histology of regeneration in skeletal muscle was provided by Waldeyer in 1865 (cited in Gilbert and Hazard, 1965). His concept of mitosis to produce a population of cells which then fuse together to form a multi-nucleate muscle fibre has been firmly established (Allbrook, 1975). Waldeyer (1865) (cited in Allbrook, 1975) termed these cells "muskelkörp erchen." In 1893, Volkman (cited in Gilbert and Hazard, 1965) suggested that these cells had a dual purpose of assisting resorption of the necrotic material and the formation of new fibres. Volkman's (1893) concept of phagocytosis has gained wide acceptance. Godman (1957) assumed the function of these cells to be primarily phagocytic although it was inherent in Volkman's (1893) concept that a certain number of these myoblasts would fuse to form the new fibre.

Zenker, in 1864 (cited in Allbrook, 1975) described muscle fibre regeneration from myoblasts placed around the necrotic fibre which give rise to myotubes which finally

become mature muscle fibres (Allbrook, 1975). Waldeyer's (1865) contemporary, Kraske (cited in Allbrook, 1975), thought the dividing nuclei came from surviving muscle nuclei, an idea that has been echoed by modern electron miscroscopic studies of the satellite cell (Mauro, 1961).

Historically, the process of regeneration was thought of as a kind of vegetative growth of fibre or an increase in number by mitotic division. The question was whether the fibres arose from new myoblasts, as was known to occur in embryonic muscle development, or had the fibres bridged a defect by growth in continuity (Allbrook, 1973)? Today's view is that both processes take place, but repair occurs in different ways depending upon the type and extent of the damage to the fibres (Allbrook, 1975).

Degeneration

Degeneration following crush injury has been reviewed by Price, Howes, and Blumberg (1964). The basement membrane remains intact and within twenty-four to forty-eight hours torn fibres become infiltrated with phagocytic cells. The time of appearance of the trauma histologically is variable according to the forces involved and mechanism of injury. However, there is gross edema, hemorrhage, and disruption of fibre integrity on longitudinal section investigation. Fibre fragmentation and obvious cell wall rupture occurs. On cross-section, the fibre bundles may be separated in a spaced pavement appearance due to edema (Smith, R., Note 1).

Nuclei appear between the basement membrane and degenerating muscle fibres. They are myoblast precursors (Carlson, 1973). There is controversy in the literature as to whether these cells originate from the degenerating muscle fibre, or are the direct descendants of satellite cells (Church, 1970; Mauro, 1961; Walker, 1963).

Process of Regeneration

Traumatized fibres show intracellular edema, loss of definition of myofilaments and widely dispersed granularity. The sarcotubular system (the endoplasmic recticulum) appears distended by edema. The mitochrondia are enlarged. After twenty-four hours, the fibres either appear normal or are undergoing phagocytosis (Allbrook, 1962).

The evidence presented by Allbrook (1962) indicated that more severely damaged necrotic fibres do not recover - either functionally or morphologically. Rather, they go through a cycle of phagocytosis and degeneration. Severely damaged fibres are edematous, there is a loss of transverse banding of the sarcomeres and the Z discs disappear. The myofibrils appear distorted or broken and their filaments disappear. The sarcoplasm appears granular, internal structure may be absent and mitochondria are enlarged. It may be that the variety of the degenerative change depend on the state of contraction of the damaged fibre at the time of histochemical fixation (Allbrook, 1962).

The sarcolemmal tube is usually intact though it may

be distorted or even collapsed. Polymorphonuclear neutrophils from the vascular system invade the area within twelve hours. Four days after injury, these cells have decreased in number. Autoradiographic studies indicate that these cells migrate from outside the area to the damaged tissue (Blintoff and Walker, 1960).

Between four and six days after injury, the sarcolemmal tubes become lined with myoblasts. Muir et al. (1964) implied that these were the satellite cells first described by Mauro in 1961. Electron microscopic investigation revealed the presence of myofilaments at this stage beneath the plasma membrane. Within a few days, myotubes appear containing bundles of myofibrils. After three weeks, near-normal size muscle fibres dominate. The fibres have their own new sarcolemmal tubes. The muscle fibres are formed by the union of individual myoblasts. Labelling of the individual muscle cells with radioactive isotopes confirms the source of the cell populations (Walker, 1963). Simultaneously surviving sarcolemmal tubes demonstrate regeneration by the same mechanism.

Along with the muscle regeneration, fibroblasts increase in number in the tissue spaces. The collagen network deposited, impedes longitudinal growth of regenerating muscle fibres. Myoblasts cannot always be identified with certainty under light microscopy (Carlson, 1973). Yet, numbers of myofibres per unit area of injured tissue have been counted (Järvinen, 1975). The number of myoblastic cells reaches a

peak between forty-eight and seventy-two hours post-trauma (Carlson, 1972). Myotubes are formed by the fusion of myoblasts (Reznik, 1968). In rabbit muscle, the lines of tension within a muscle probably determine the orientation of regenerating fibres (Allbrook, 1951).

Mincing the muscle as a form of injury has demonstrated that mechanical tension is the primary factor leading to the internal organization of muscle. The tension is exerted via the interstitial connective tissue which is formed in the regenerating process (Carlson, 1971, 1972).

The moulding of regenerating muscle seems to be due to two factors:

1. the area of regenerating fibres decreased in mass due to scar consolidation
2. the area of massive trauma pressure of surrounding tissues moulds the fibre.

Carlson (1972) stated that mechanical tension appeared to be the main force directing the internal organization of regenerating muscle. Mechanical tension serves to orientate collagen fibres along the lines of tension. Such collagen fibres harness the muscle fibres serving to transmit tension from connective tissue to repairing muscle. Tension is a stimulus for rapid and greater myofibrillar deposition (Allbrook, 1966; Carlson, 1972). Allbrook (1966) believed that it was likely that persistent tissue space edema and excessive collagen deposition were closely associated.

Manual Manipulative Therapy of the Soft Tissues

Dr. James Cyriax described his latest treatment technique of "frictions" to soft tissues in 1969. Placing the traumatized soft tissue on stretch through positioning, the technique involved manual therapy using fingers and thumbs rhythmically oscillating across the fibre direction to a pressure of discomfort. Millar (1976) described a technique for calf self-stretch up to the point of discomfort. The treatment was commenced forty-eight hours following closed intrinsic trauma.

The treatment technique used in the current study incorporated thumb pressures applied through the skin to traumatized muscle fibres, with the muscle belly relaxed. Rhythmically compressing the tissue between underlying osseous skeleton and thumbs, or using fingers for counter-pressure to the thumbs, where appropriate to muscle bulk, tension can be applied very locally to healing fibres to facilitate repair. Pressure is to the point of discomfort. The direction and amount of pressure applied can be varied according to the discomfort and degree of muscle spasm produced. Thumbs and fingers do not move across skin, rather they press into it to effect underlying muscle such that applied pressure is rhythmically reduced and increased without loss of skin contact with fingers and thumbs. No evidence was found in the literature for the effects of such specific, localized treatment.

Chapter 3

METHODOLOGY

Experimental Design

The concept of the study was based on histological investigation of treated and untreated muscle. By injuring both hind limbs of six of the seven rabbits identically, it was then possible to assign one as treatment leg, and the other as injured control for each rabbit. The advantage of this design was that each rabbit acted as its own control. Furthermore, by treating one limb with manual soft tissue manipulation, it was possible to compare the muscle regeneration of treated versus untreated muscle at assigned days of three, seven, ten, fifteen and twenty-one, post-trauma. Thus a histological spectrum of healing rate for treated and untreated muscle was obtained.

Implicit in the design was the need to administer impact trauma of identical magnitude to all medial gastrocnemii of the six rabbits. To achieve this, it was necessary to standardize trauma, and determine appropriate impact. To determine aspects of the rate and process of regeneration in the rabbit muscle, a preliminary study, which also served to determine the histological spectrum of normal regeneration after injury, was conducted.

Subjects

Seven male, New Zealand white rabbits, aged 1.5 to 2.5 years and of similar body weights (Table 1) were supplied by the breeder for the investigation. Each animal was placed in a cage, 40 cm x 60 cm x 40 cm. "Master" brand food and water were provided without restriction.

Anaesthesia

Anaesthesia was administered using a Fluotec 3 unit (Plate 1). One hundred percent oxygen was used to drive the halothane and a canvas restraining bag with head opening was used during the induction of the anaesthetic to limit the rabbit's movement. Induction and maintenance of anaesthesia was via a mask encompassing nostrils and mouth (Plate 2).

Induction flow was measured from the equipment at five litres per minute one hundred percent oxygen, and drove the five percent halothane. The rabbit was considered to be adequately under the effect of the anaesthesic when the pupils had become fixed and dilated, respiration was regular and deep, and there was no active response to passive movement of the limbs. At this stage, halothane concentration was reduced to three percent in order to maintain anaesthesia, and the one hundred percent oxygen reduced to a flow rate of three litres per minute.

Table 1

SUBJECT AND TREATMENT DATA

Subject Number	Body Weight (Grams)	Trauma to Both Legs	Leg Treated	Number of Treatments	Surgery for Histology	Distance From Trauma Site to Heel (Left Leg)	Distance From Trauma Site to Heel (Right Leg)
1	3410 gm	Day 1	No Treatment	0	Day 2	9.41 cm	9.32 cm
2	3950 gm	Day 1	Right	1	Day 3	9.18 cm	9.27 cm
3	3860 gm	Day 1	Right	3	Day 7	9.23 cm	9.40 cm
4	3856 gm	Day 1	Right	6	Day 10	9.36 cm	9.28 cm
5	3910 gm	Day 1	Left	9	Day 15	9.24 cm	9.26 cm
6	3756 gm	Day 1	Right	9	Day 21	9.28 cm	9.18 cm
7	3430 gm	No Trauma Inflicted	No Treatment	0	Day 22	No trauma inflicted. Distance from normal sample site to heel, left leg 9.59.	No normal sample taken from right leg.

Plate 1. Rabbit under halothane anaesthesia maintenance following induction.

Plate 2. Induction of halothane anaesthesia via mask.

Point of Impact

A Toledo scale was used to measure body weight of the anaesthetized rabbit. Oster animal clippers were then used to shave both hind limbs of the rabbit from the heel to above the knee. Mimmotu calipers measured the distance from the proposed point of impact on the medial head of gastrocnemius to the heel at the tip of the Os calcis (Plate 3). Positioning for measurement was ninety degrees knee flexion and ninety degrees ankle dorsiflexion (Plate 3).

Plate 3. Calipers measuring from proposed impact point to the heel. Rabbit under anaesthesia and positioned.

Infliction of Trauma

A hollow aluminum cylinder with an internal diameter of 2.10 cm was positioned with its inferior opening directly

over the muscle site by means of clamps and stand to ensure its verticality. The cylinder was directly over the mark on the skin with the limb positioned as described above, the rabbit in sidelying (Plate 4). This position was passively maintained under anaesthesia manually, the care was taken to see that the medial border of the tibia was positioned just outside the cylinder's opening to prevent its fracture. A steel block, 4.0 cm high, was placed between the calf complex and the table, so as to provide a tight calf compartment and ensure no deflection of the injuring weight.

A solid steel cylinder with a diameter of 1.90 cm, 35.6 cm long, and weighing 731.0 grams, with a hemispherical inferior tip radius of curvature 0.95 cm, was introduced into the top of the hollow cylinder. It came to rest against the cross pin at 55.5 cm above the muscle. The removal of the pin effected release of the cylinder.

Soft Tissue Manipulation

The rabbit was suspended in a restraining bag with the injured limb to be treated hanging free from the zippered inferior opening. This limb quickly relaxed and it was possible to treat the limb with manual soft tissue manipulative therapy with the rabbit fully conscious. With some individuality, it was found that rabbits soon accommodated to, and tolerated, the treatment (Plate 5).

A treatment of one hundred and sixty bilateral thumb pressures per minute administered for five minutes continually

Plate 4. Rabbit under anaesthesia. Equipment positioned for infliction of trauma to left medial head of gastrocnemius, with rabbit in left sidelying.

Plate 5. Conscious rabbit suspended in restraining bag with right hind limb to be treated, freely suspended from bag.

constituted one treatment. Such treatment was carried out in the final trial with the six rabbits daily, from day two after injury. The investigator's index and middle fingers rested on the skin covering the lateral aspect of the lateral head of gastrocnemius and provided counter-pressure for the manipulating thumbs, which pressed directly into the muscle.

Immediately after cessation of the treatment, the manipulation was transferred to the cuff of a sphygmomanometer inflated to 20 mm Hg to simulate the muscle tone. The maximum pressure registered after thirty seconds of recommenced manipulation was recorded from the sphygmomanometer.

Tissue Samples

Tissue samples were taken at one, three, seven, ten,

fifteen, and twenty-one days, post-injury. The animals were sacrificed by administering pentobarbitol directly into the heart, via hypodermic needle. Immediately after death, the mid-belly of the muscle in both legs were exposed and removed, using forceps and scalpel. To ensure removal of injured tissue, the calipers were again used with the limb positioned as at the time of injury to relocate the exact epicentre of the injury (Plate 6).

Plate 6. Removal of injured muscle tissue.

The section of gastrocnemius muscle was removed surgically to the full depth of the medial head, and as far proximally and distally as possible to include all injured tissue corresponding to the diameter of the injuring cylinder, and a periph-

eral area of potentially uninjured tissue.

Histology

Tissue fixation and washing. The tissue was marked with Indian ink dye on its injured medial surface and immediately transferred to a container of ten percent formalin ten times its volume, in order to preserve the cells (Humason, 1979). The container was labelled with date, rabbit number and leg. At the time of histological investigation, the tissue was first transferred to fifty percent alcohol (ethanol) to wash it free of formalin and allowed to stand for six hours.

Dehydration and clearing. To adequately section the material, it was necessary to dehydrate it and embed it in paraffin. Dehydration was achieved by immersing the tissue in a series of solutions of ethyl alcohol in water, with increasing percentages of alcohol from thirty percent through to absolute alcohol. Because the alcohol used for dehydration would not dissolve the paraffin, it was necessary to "clear" the tissue for paraffin embedding as an intermediate step, with xylene.

Paraffin infiltration and embedding. Because sections as thin as six μ (1/6000 mm) were to be prepared, paraffin of melting point (56 - 68°C) was used. The tissue was transferred from cleaning agent to paraffin, the oven temperature being kept just high enough to maintain the paraffin in a just-melted state.

The tissue was placed directly in the block contain-

ing melted paraffin and orientated so that serial longitudinal section could be obtained, cutting parallel to the plane of the medial aspect of the belly.

Sectioning. The rotatory microtome was set for a section thickness of six μ and the block clamped in position. The knife blade was inserted, and its cutting edge tilted toward the block. A "ribbon" of tissue was then cut. Fifteen serial sections were retained. After floating these in a water bath, set at a temperature below the paraffin's melting point, the sections were transferred to slides and cover slips positioned, mounting with "Permount" solution.

Staining

Haematoxylin, when oxidized, stains chromatin blue-black (Humason, 1979). Mallory's haematoxylin was used and stained nuclei deep purple. Alcohols were used to control the intensity of staining. Eosin staining which stained all tissue except the nuclei, shades of pink, then followed.

Masson's trichrome staining technique which includes four dyes, iron alum, haematoxylins, acid fuschsin, and Ponceau de xylidine (Humason, 1979), then followed. The first two stains require one half hour each for fixation, washing in water between steps. Acid fuschsin and Ponceau de xylidine require one to five minutes' washing in distilled water and dehydrating alcohols. The trichrome serves to stain connective tissue, collagen, and fibroblasts, shades of green.

Investigation

The slides were examined using a Zeiss light microscope. Colour photographs of histological changes over the twenty-one days for injured treated and non-treated tissue were taken with the Zeiss camera unit attached to the microscope. Ektachrome 160 tungsten filament film was used. Fifteen slides of each tissue were examined. Tissue was also taken from the lateral side of the medial head of gastrocnemius to demonstrate that the trauma was not histologically evident to that depth.

Photographs were taken by the investigator at power 400 after examining the tissue at lower power. The tissue was not labelled on the slide as treated or untreated tissue, to eliminate any bias by an investigator when selecting a site on the slide for high power photographs.

Chapter 4

RESULTS

Response to Injury

None of the rabbits showed any visible response to the injury. They paid no particular attention to the trauma sites, showed no alteration in eating and/or drinking habits, and cage activity was unchanged from that prior to injury.

Ease of Treatment

Within slight individual behavioural temperaments, the rabbits were easy to treat and became moreso with increased handling.

Cuff Pressure Results

Treatment pressures used by the investigator were confined to levels short of developing muscle spasm and sufficient tenderness for the rabbit to withdraw the limb. The pressures recorded were those registered on the sphygmomanometer after treatment.

Normal Tissue (Rabbit #7)

Plate 7 demonstrates the cross banding of the myofibrils of the normal tissue in longitudinal section. Nuclei were demonstrated to lie peripherally placed under the sarcolemma. Fibres were in close aposition running parallel to each other.

COLOURED PICTURE

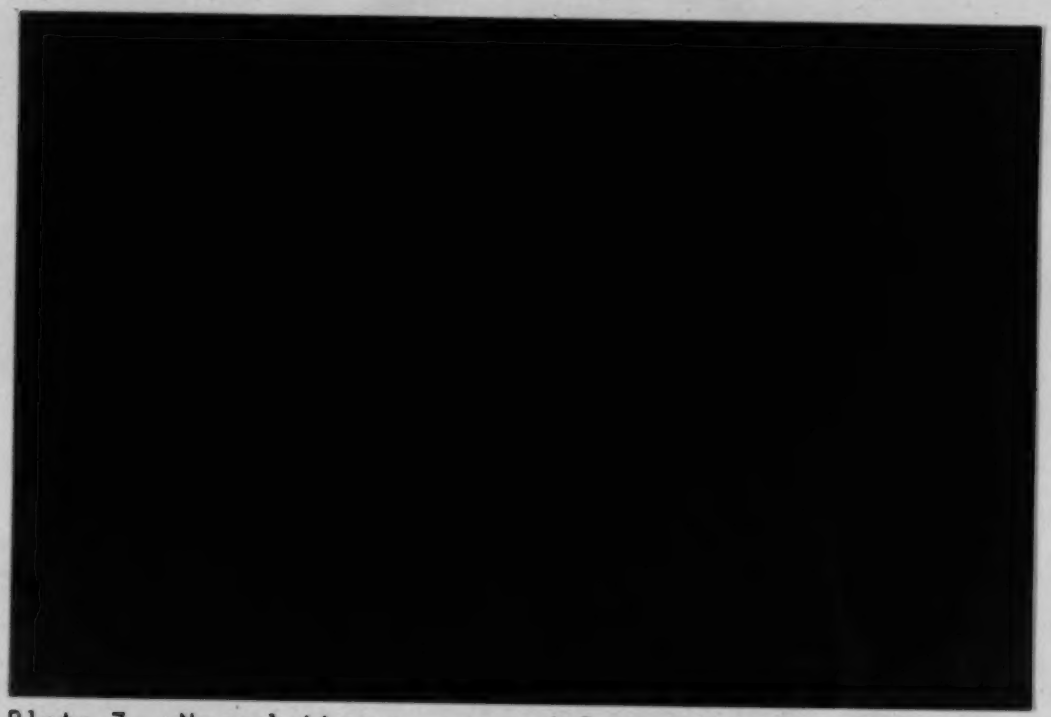

Plate 7. Normal tissue, removed from rabbit #7. Haematoxylin and eosin stain, x 400.

Twenty-Four Hours Post Injury (Rabbit #1)

Plate 8 demonstrates fibre disruption in untreated tissue with haematoxylin and eosin stain. Some loss of banding and the presence of nuclei situated randomly in the injured muscle showed characteristic trauma. The separation of fibres due to edema was also noted.

Plate 9 reveals the blue-green trichrome stain in the region of fibre disruption, taken from rabbit #1.

Three Days Post Injury (Rabbit #2)

Untreated tissue (Plate 10) demonstrated fibre disruption and the presence of numerous nuclei and macrophages

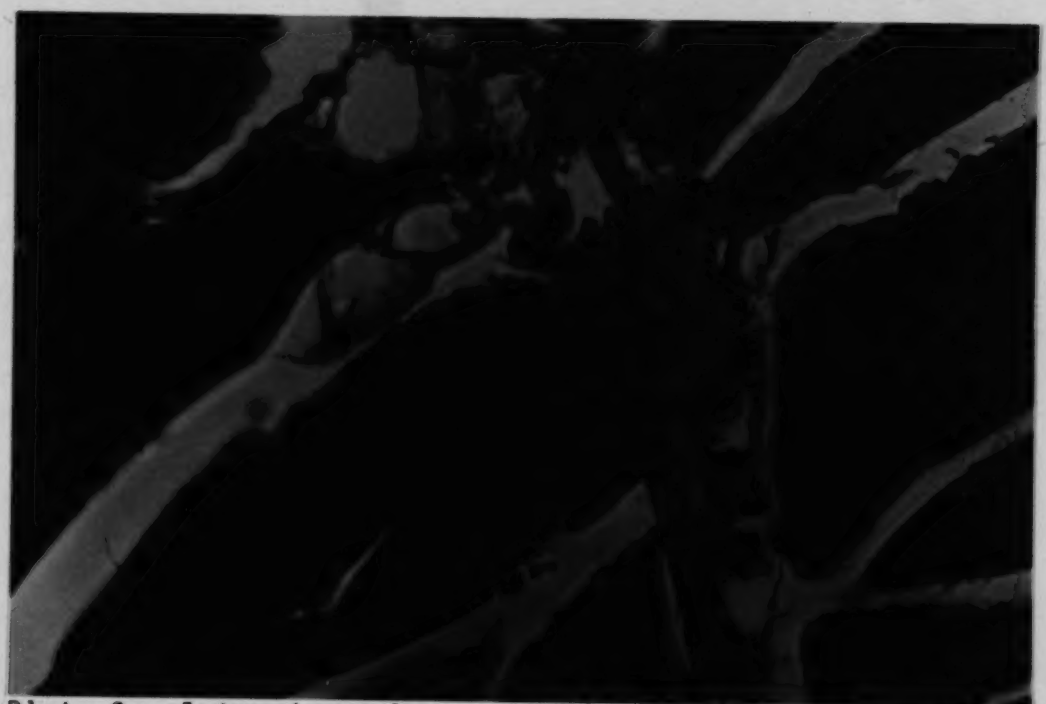

Plate 8. Injured muscle removed from rabbit #1, twenty-four hours after injury. Haematoxylin and eosin stain, x 400.

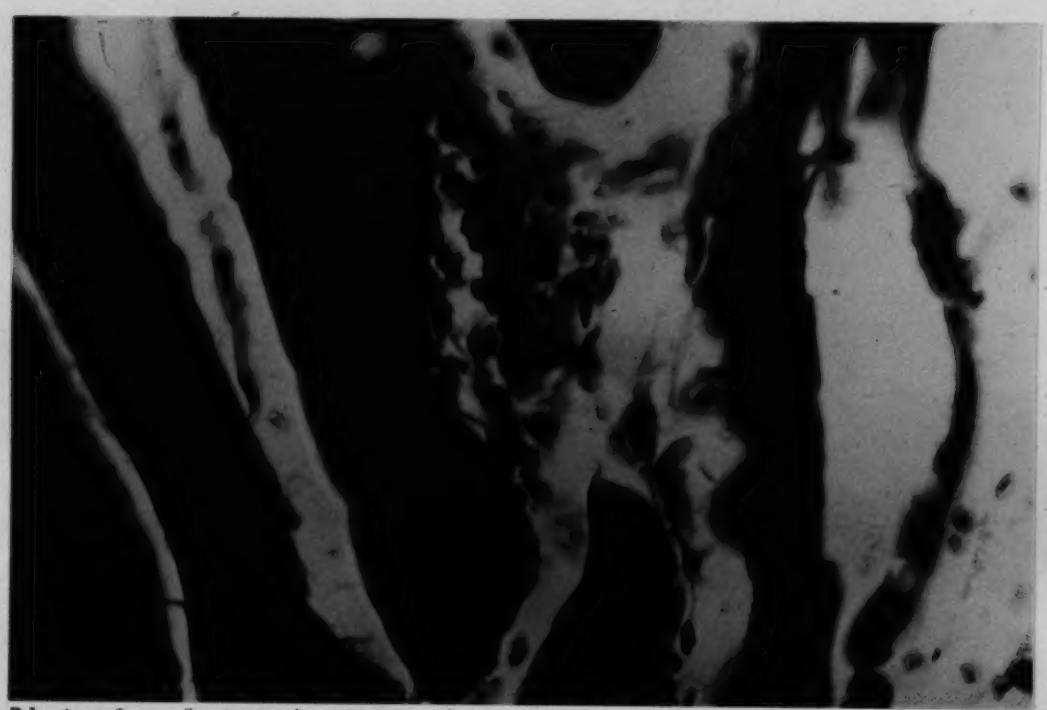

Plate 9. Injured muscle from rabbit #1, twenty-four hours after injury. Trichrome stain, x 400.

COLOURED PICTURE

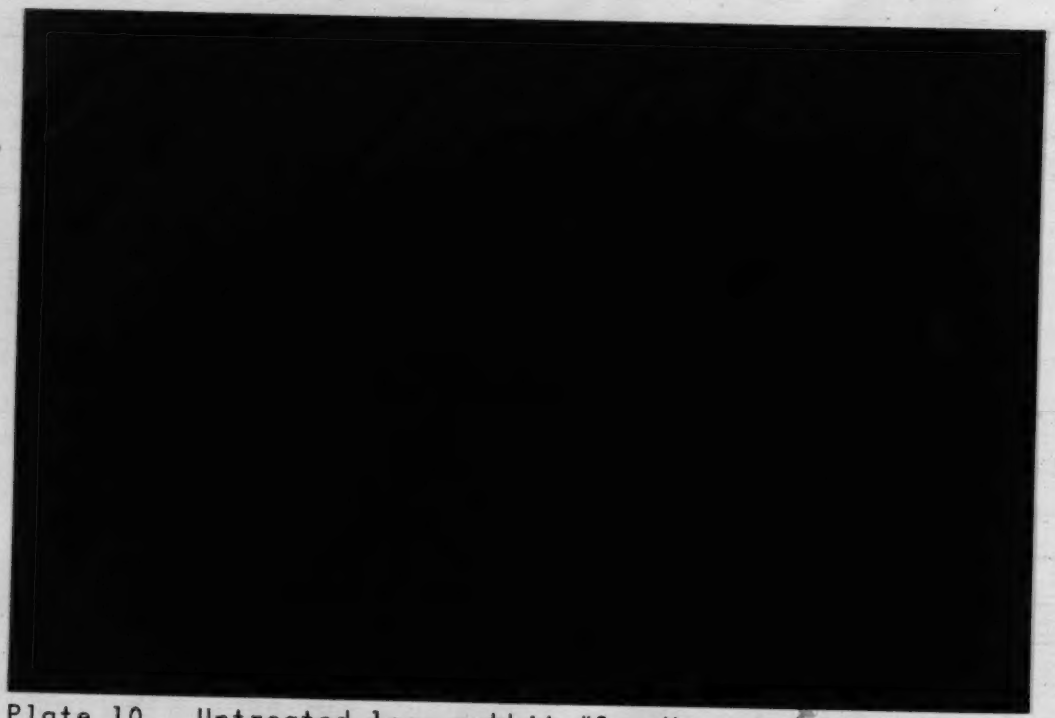

Plate 10. Untreated leg, rabbit #2. Haematoxylin and eosin stain, x 400.

The treated tissue was of similar appearance (Plate 11).

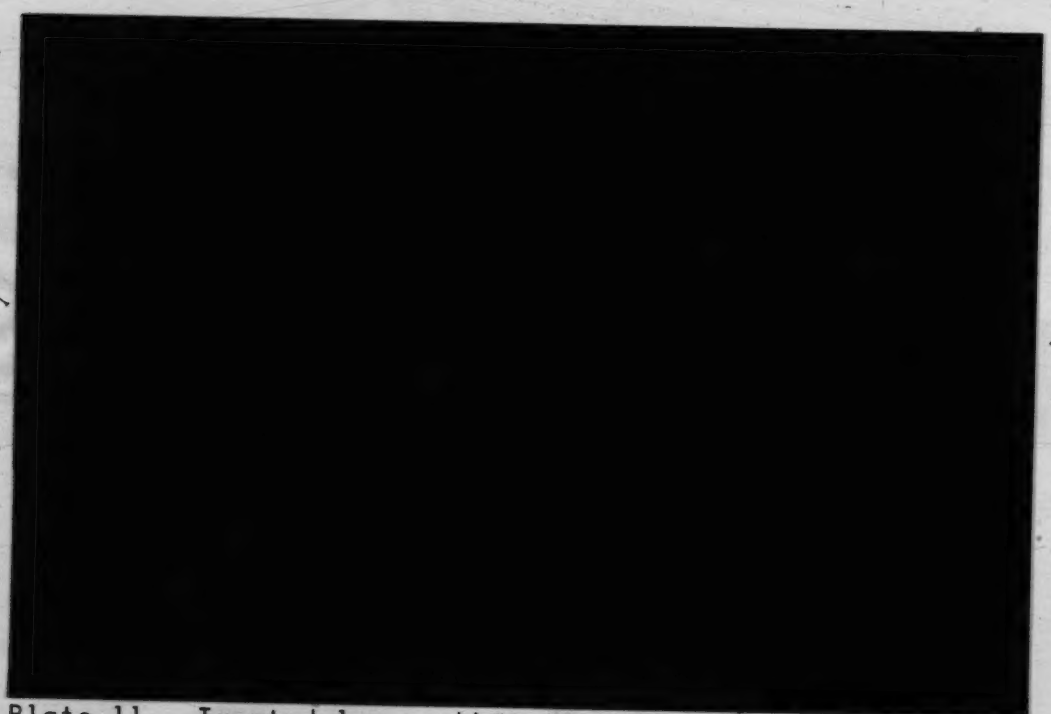

Plate 11. Treated leg, rabbit #2. Haematoxylin and eosin stain, x 400.

COLOURED PICTURE

The trichrome stain of treated tissue demonstrated substantial collagenous tissue staining between separated and disrupted fibres (Plate 12). The untreated tissue gave a less disrupted appearance (Plate 13).

Seven Days Post Injury
(Rabbit #3)

The treated muscle (Plate 14) demonstrated proliferation of nuclei (presumptive myoblasts) showing disorganized orientation. Edema was reduced, the fibres now appearing more aposed. Cross banding was visible. Nuclei were beginning to adopt peripheral organization. The untreated muscle (Plate 15) demonstrated numerous nuclei also, but they appeared to be in a less advanced stage of organization as evidenced by their disorientation within the fibres. Edema was reduced.

Ten Days Post Injury
(Rabbit #4)

Untreated muscle with haematoxylin and eosin staining showed nuclei beginning to become peripherally orientated within the fibres (Plate 16). The untreated tissue (Plate 17) appeared unchanged from seven days post injury. Both treated and untreated tissue showed close fibre aposition implying minimal edema between fibres. The trichrome staining of both treated and untreated muscle now demonstrated approximately equal inter-fibre scarring.

Fifteen Days Post Injury
(Rabbit #5)

The treated tissue demonstrated organized and more

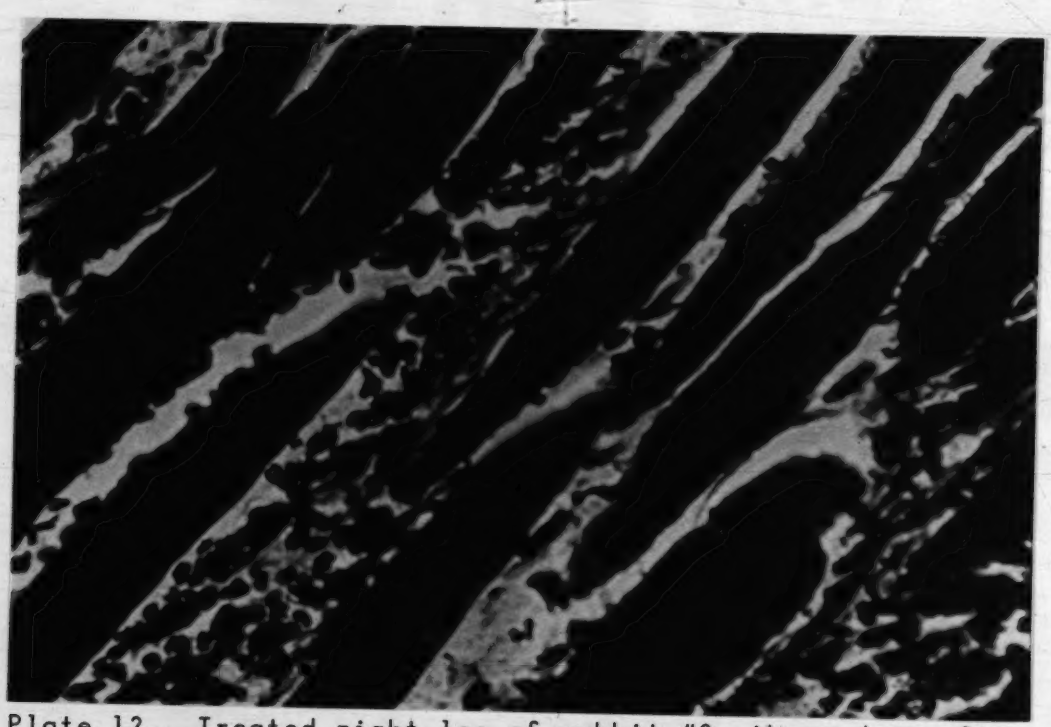

Plate 12. Treated right leg of rabbit #2, three days after injury. Trichrome stain x 400.

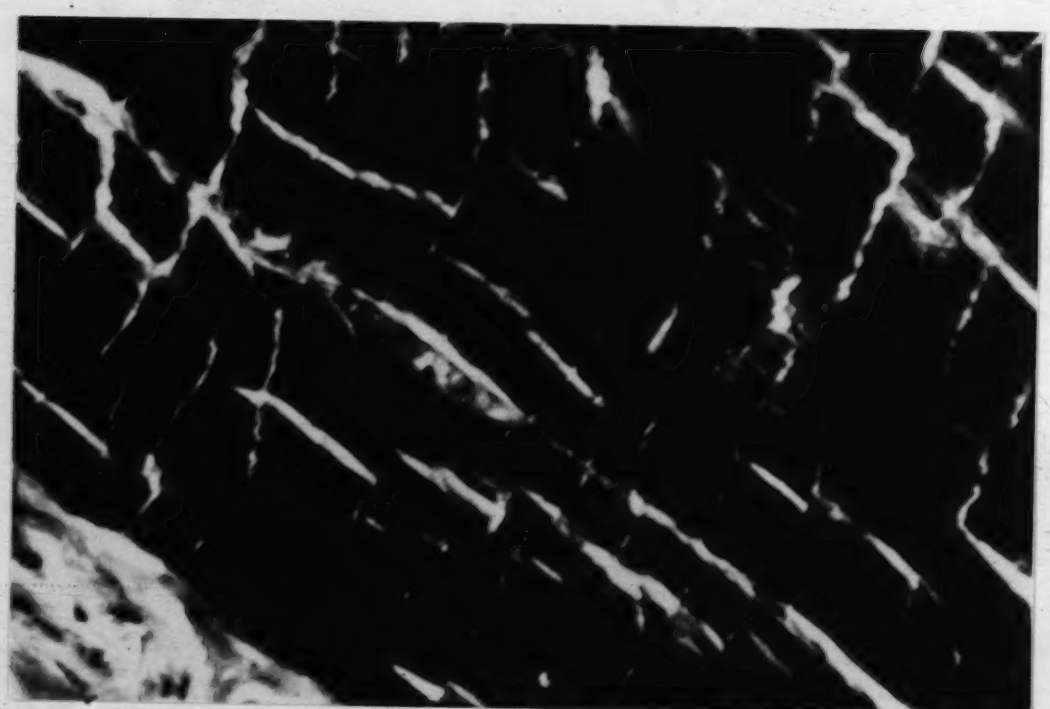

Plate 13. Untreated left leg of rabbit #2, three days after injury. Trichrome stain, x 400.

Plate 14. Treated right leg of rabbit #3, seven days after injury. Haematoxylin and eosin stain, x 400.

Plate 15. Untreated left leg of rabbit #3, seven days after injury. Haematoxylin and eosin stain, x 400.

COLOURED PICTURE

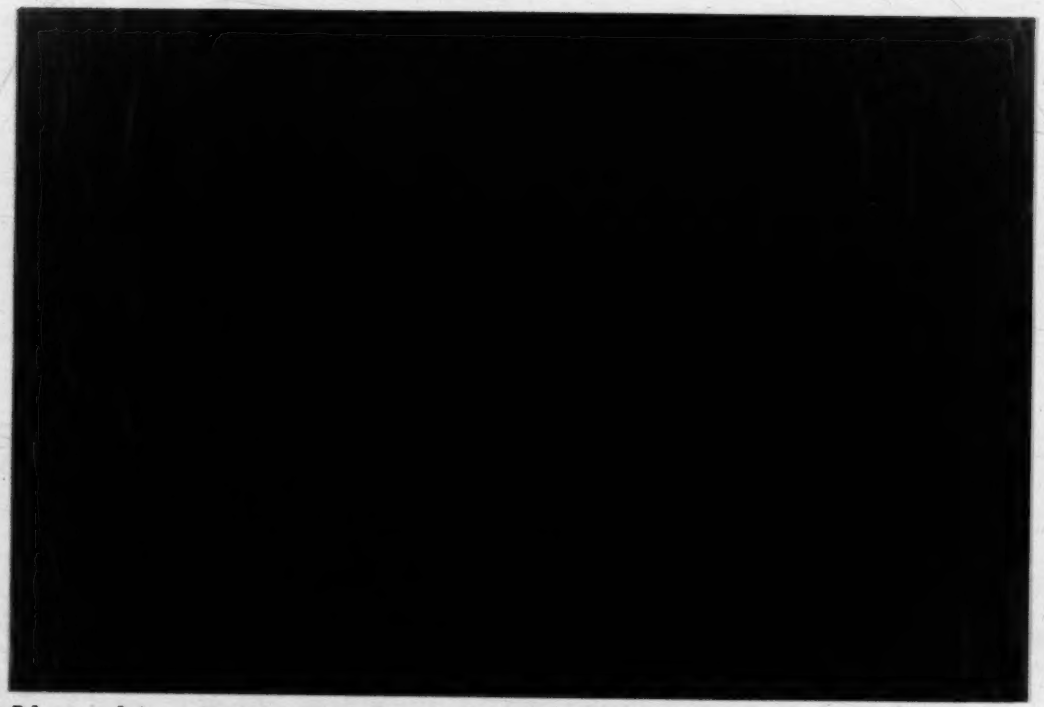

Plate 16. Untreated leg of rabbit #4, ten days after injury. Haematoxylin and eosin stain, x 400.

Plate 17. Treated leg of rabbit #4, ten days after injury. Haematoxylin and eosin stain, x 400.

COLOURED PICTURE

peripherally orientated nuclei at this time (Plate 18). The untreated muscle (Plate 19) was less advanced in this respect. No edema was seen at this time in either treated or untreated muscle.

The treated tissue demonstrated slightly reduced scarring compared to untreated tissue. There was no significant difference in scarring between treated and untreated tissue as shown by trichrome stain.

Twenty-One Days Post Injury
(Rabbit #6)

All treatment ceased after fifteen days post injury. At twenty-one days post injury, the treated tissue (Plate 20) appeared as normal tissue compared to the normal control tissue taken from the uninjured rabbit #7. The untreated muscle (Plate 21) nuclei were still somewhat disorientated. No edema was present. The extent of collagenous tissue was substantially reduced by day twenty-one. However, there was no significant difference between treated and untreated tissue in this respect.

Summary of Results

Table 2 presents a summary of the results for treated and untreated muscles for the seven rabbits, and Table 3 illustrates the post treatment cuff pressure differentials.

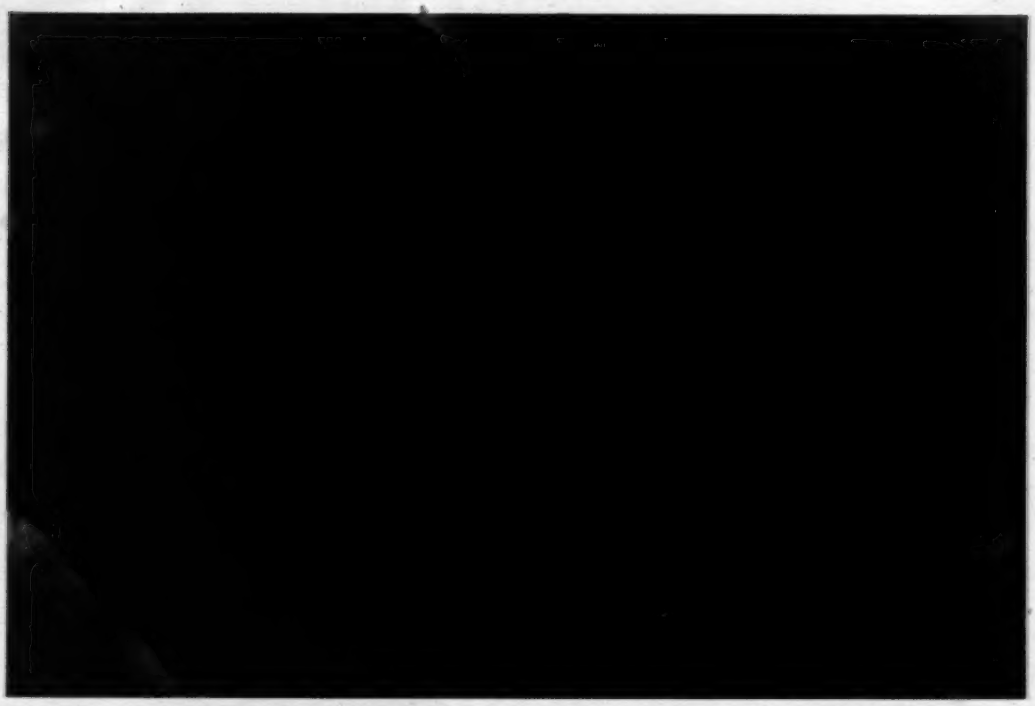

Plate 18. Treated leg of rabbit #5, fifteen days after injury. Haematoxylin and eosin stain, x 400.

Plate 19. Untreated leg of rabbit #5, fifteen days after injury. Haematoxylin and eosin stain, x 400.

COLOURED PICTURE

Plate 20. Treated leg of rabbit #6, twenty-one days after injury. Haematoxylin and eosin stain, x 400.

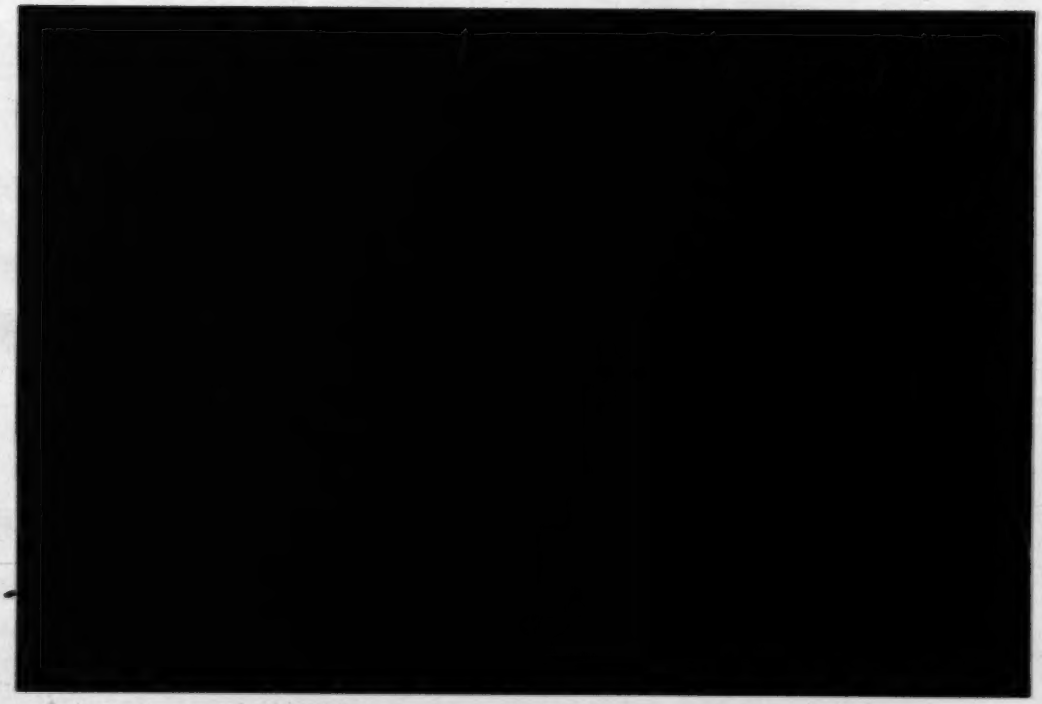

Plate 21. Untreated leg of rabbit #6, twenty-one days after injury. Haematoxylin and eosin stain, x 400.

COLOURED PICTURE

Table 2

SUMMARY OF RESULTS

Days Post Injury	UNTREATED MUSCLE			TREATED MUSCLE		
	Edema	Haematoxylin and Eosin Staining	Trichrome Staining	Edema	Haematoxylin and Eosin Staining	Trichrome Staining
24 Hours Rabbit #1	Fibre disruption and separation	Increased nuclei, randomly situated. Loss of banding.	Presumptive fibroblastic activity between fibres.	Tissue not treated. No difference in appearance to control leg.		
3 Days Rabbit #2	Reduced edema	Fibre disruption. Macrophages. Fibre degeneration.	Collagenous staining between separated fibres.	Reduced edema	Fibre disruption. Macrophages. Fibre degeneration.	Substantial collagen staining between tissues.
7 Days Rabbit #3	Minimal edema	Proliferation of nuclei. Disorganization.	Reduced collagen.	Minimal edema	Proliferation of nuclei. Disorganization.	Reduced collagen.
10 Days Rabbit #4	Minimal edema	Proliferation of nuclei. Disorganization persisting.	Scarring between fibres.	Minimal edema	Proliferation of nuclei. Disorganization resolving.	Scarring between fibres.
15 Days Rabbit #5	No edema	Nuclei organizing.	Scarring unchanged.	No edema	Nuclei organized and orientated.	Slightly reduced scarring.
21 Days Rabbit #6	No edema	Nuclei still orientating.	Scarring reduced overall.	No edema	Tissue normal.	Scarring reduced overall.
Uninjured Tissue Rabbit #7	N O R M A L T I S S U E					

Table 3

POST TREATMENT CUFF PRESSURE DIFFERENTIALS

Subject Number	Treatment (Rx) 1 Day 3	Rx 2 Day 4	Rx 3 Day 7	Rx 4 Day 8	Rx 5 Day 9	Rx 6 Day 10	Rx 7 Day 11	Rx 8 Day 14	Rx 9 Day 15	Day 21	Day 22
1	Surgery Day 2										
2	20 to 38 mm Hg	Surgery									
3	20 to 40 mm Hg	20 to 44 mm Hg	20 to 48 mm Hg	Surgery							
4	20 to 42 mm Hg	20 to 44 mm Hg	20 to 48 mm Hg	20 to 46 mm Hg	20 to 44 mm Hg	20 to 48 mm Hg	Surgery				
5	20 to 44 mm Hg	20 to 46 mm Hg	20 to 46 mm Hg	20 to 48 mm Hg	20 to 48 mm Hg	20 to 50 mm Hg	20 to 48 mm Hg	20 to 48 mm Hg	Surgery		
6	20 to 44 mm Hg	20 to 48 mm Hg	20 to 52 mm Hg	20 to 50 mm Hg	20 to 46 mm Hg	20 to 48 mm Hg	20 to 48 mm Hg	20 to 50 mm Hg	No Treatment Day 15 to Day 21	Surgery	
7											Surgery

Chapter 5

DISCUSSION

A variety of injuring devices have been reported in the literature (Allbrook, Baker, and Kirkaldy-Willis, 1966; Järvinen and Sorväri, 1975), but none of these was adequate for use in the present study and none had involved the New Zealand white rabbit. There have also been methods developed for the production of experimental ischaemia (Mäkitie and Teräväinen, 1977; Reznik, 1969). Very severe crush injuries have been developed, as reported by Allbrook (1962) and Church (1966). A major concern of the present investigation was to devise a method of injury appropriate to the New Zealand white rabbit and one that produced closed injury, with an epicentre of trauma and the skin remaining intact. Such trauma had to be of sufficient magnitude to resemble an impact injury in a human, yet not so great as to cause more than discomfort for the animal when conscious.

In developing an appropriate method, the impact weight was dropped from successively greater heights until evidence of skeletal muscle trauma was observable at surgery, twenty-four hours post injury. Bleeding within the fascial sheath and fibre discolouration were readily observable at this time. Further to this, the muscle was tender on palpation. There was no loss of normal limb function and the animals

ignored their injuries, maintaining normal cage activity.

During impact, it was necessary to keep the tibia outside of the direct impact area in order to eliminate the possibility of fracture. A block under the muscle belly prevented the impact implement from deflecting across the skin and ensured that most of the energy was absorbed by the mid-belly of the muscle. The energy dissipated on impact was calculated to be 40×10^6 dyne cm. No allowance was made for friction between weight and cylinder as the weight fell.

It was observed that the rabbits would not withdraw the suspended limb repeatedly during soft tissue manipulation. Rather, they quickly became accustomed to this therapy and it was possible to administer up to five minutes of minimally interrupted treatment. A sphygmomanometer cuff of 20 mm Hg pressure was used to represent the relaxed rabbit muscle tone. Thus, pressure transfer from muscle to cuff gave a good estimate of the pressure applied to the muscle by the investigator's hands during the immediately preceding treatment.

Anatomical variations in the animals and variations in depth of anaesthesia can affect the degree of trauma resulting from a constant blow at the site of the trauma. Variations in the location of the injury affect histological outcomes. These are variables that are difficult to accurately control. Further, Allbrook (1962) highlighted the possible complicating effect of any state of contraction or fibrillation of the damaged fibres at the time of excision. Järvinen and Sörväri's (1975) injury to female Wistar rats of average body weight

219.5 gm, dissipated 4.5×10^6 dyne cm average to gastrocnemius, compared with 40×10^6 dyne cm in this investigation with male white New Zealand rabbits of average body weight 3790.3 gm. The former injuries were more severe to the muscle.

Effects of Soft Tissue Manual Treatment

Edema. From twenty-four hours after injury, both treated and untreated tissue demonstrated fibre disruption and separation consistent with edema and inflammation. Three days post injury, edema between fibres was reduced in both treated and untreated tissue. At seven days post injury, edema was minimal and by fifteen days post injury, both treated and untreated tissue did not demonstrate any edema.

Haematoxylin and eosin staining. The injured tissue demonstrated increased numbers of nuclei randomly situated, with loss of banding by twenty-four hours after injury. Three days after injury, both treated and untreated tissue showed fibre disruption with the presence of macrophages indicating phagocytosis of degenerating tissue. At seven days after injury, there was further proliferation of disorganized nuclei (satellite cells and myoblasts). By ten days after injury, the untreated tissue demonstrated disorganization of nuclei persisting. The treated tissue appeared to show slightly more resolution of repair with the nuclei beginning to adopt peripheral orientations within the fibre myotube. At fifteen days post injury, the treated tissue was organized and orientated. By twenty-one days after injury, the treated tissue

resembled the normal tissue, while the untreated tissue nuclei were still undergoing peripheral orientation.

Trichrome staining. Fibroblastic activity between fibres was demonstrated from twenty-four hours after injury. At three days after injury, the treated tissue demonstrated more substantial collagenous staining and fibroblastic activity than did the untreated tissue. It is speculated that early treatments may evoke increased collagenous activity at this stage. At seven days post injury, both treated and untreated tissue demonstrated reduced fibroblastic activity. At ten days, both sets of tissue appeared similar demonstrating scarring between fibres. At day fifteen post injury, there was slightly reduced scarring compared with the untreated tissue. Beyond this time, the scarring was significantly reduced but remained unchanged between treated and untreated tissue.

Manual Soft Tissue Treatment

No method was found in the literature which described the treatment and the method of administering a relatively objective reproducible "dosage" of soft tissue manipulation. Although the method of using a sphygmomanometer cuff to reflect pressure of manipulating fingers and thumbs was indirect, it was the most practical and feasible technique available in this situation. Traumatized muscle treated by the method of soft tissue manipulation readily goes into spasm if treatment is excessive. Thus, treatment pressure short of developing muscle spasm and sufficient tenderness for the rabbit to with-

draw the limb, was administered. Having subsequently recorded pressures, it was of interest to note that as daily treatments progressed, it was appropriate to apply more thumb pressure up to 52 mm Hg with no subsequent spasm or withdrawal occurring. This finding was consistent with resolution of edema in the first few days (Carlson, 1973), subsequently rendering the injured tissue less tender to more vigorous manipulation of the tissue.

Relevance of the Study

Trauma is common in "contact" collision sport, and physicians and physiotherapists are confronted with rehabilitation of such injured athletes regularly. Soft tissue trauma resulting from severe impact forces consistent with vehicle accidents also necessitates carefully controlled rehabilitation of patients. Treatment methodologies that have as their basis a demonstrable therapeutic effect on muscle healing are valuable. If such treatments can be modified consistently with advances in the healing process, functional outcomes are likely to be enhanced. Soft tissue manual manipulation as outlined, applied to human skeletal muscle, is reproducible and readily modifiable, and can have demonstrably therapeutic effects on muscle repair when judiciously and advisedly administered as an integral aspect of treatment.

Chapter 6

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

From this study, it was concluded:

1) that the repair of New Zealand white rabbit skeletal muscle followed a similar histological progression and rate as outlined in the literature for human skeletal muscle (Allbrook et al., 1966; Blintoff and Walker, 1960; Carlson, 1973; Muir et al., 1965; Price et al., 1964).

2) that New Zealand white rabbit gastrocnemius muscle injured in the manner outlined in this investigation and treated by the method of soft tissue manual therapy outlined demonstrated a more rapid loss of edema and consequent fibre reaposition and parallel orientation than untreated tissue by fifteen days after injury.

3) the tissue treated, as outlined, demonstrated histologically a more rapid proliferation and subsequent reorganization of cells and repairing fibre nuclei.

Recommendations

Three significant recommendations evolve from this investigation:

a) Injury - A more forceful blow to the limb may more strongly differentiate treated as compared to untreated

tissue, histologically.

b) Electron Microscopy - It is recommended that from further tissue samples, grids be prepared for electron microscope investigation. Carlson (1973) stated that under light microscopic investigation, accurate counts of myoblasts was not possible; however, electron microscopic study would allow for the collection and analysis of numerical data and give a clearer indication of the quality of repair.

c) Electromyography and Tensiometry - Muscle tension analysis following release of the tendon of insertion may be complemented by electromyographic studies. Two micro E.M.G. electrodes may be placed under the fascia of the gastrocnemius; proximal and distal to the trauma site. Surgical exposure of the branch of the nerve to the head of gastrocnemius, between it and the lateral head, allows for attachment of very fine steel wire stimulating electrodes. These may then connect to the nerve-muscle stimulator.

Detaching the Achilles from its insertion, or the Os calcis itself, this in turn may be attached to a tensiometer, and the rabbit's limb firmly fixed skeletally. Using the nerve-muscle stimulator with electrodes attached to the exposed nerve branch to the medial head, data on the tension generated at supramaximal stimulation can be gathered, provided the stimulating electrode holds the nerve free of all underlying muscle. Further, E.M.G. readings can be simultaneously taken using a camera attached to the equipment so as to synchronize with the maximal stimulation to the nerve. Supramaximal

tensiometry and electromyography readings can then serve as the basis for comparison of healing emphasizing functional differences in the healing of injured and uninjured tissue.

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REFERENCE NOTES

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APPENDICES

APPENDIX A

Approval Letter from Ethical Review Committee

INTER-DEPARTMENTAL CORRESPONDENCE

TO David James
Graduate Student
Department of Physical Therapy

DATE April 22, 1980

FROM B. Fifield
Associate Professor & Acting Dean
Chairman, Ethical Review Committee
Faculty of Rehabilitation Medicine

Re: PROPOSAL ON MANIPULATIVE THERAPY SUBMITTED FOR ETHICAL REVIEW

At their meeting on April 18th, the Committee approved the above proposal.

The Committee wishes me to convey to you that the information submitted would not have been sufficient, had several Committee members not been familiar with your proposal. It is requested, therefore, that any future proposals be submitted in fuller detail.

Thank you.

B. Fifield

BF/ra

cc: Ethical Review file